ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Blood Lead Concentration and Thyroid Function during Pregnancy: Results from the Yugoslavia Prospective Study of Environmental Lead Exposure

Linda G. Kahn, Xinhua Liu, Biljana Rajovic, Dusan Popovac, Sharon Oberfield, Joseph H. Graziano, and Pam Factor-Litvak

http://dx.doi.org/10.1289/ehp.1307669
Received: 19 September 2013
Accepted: 22 May 2014
Advance Publication: 27 May 2014

NIH National Institute of Environmental Health Sciences

Blood Lead Concentration and Thyroid Function during Pregnancy: Results from the Yugoslavia Prospective Study of Environmental Lead Exposure

Linda G. Kahn,[1] Xinhua Liu,[2] Biljana Rajovic,[3] Dusan Popovac,[4] Sharon Oberfield,[5] Joseph H. Graziano,[6] and Pam Factor-Litvak[1]

[1]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA; [2]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York, USA; [3]Regional Hospital Kosovska Mitrovica, Mitrovica, Kosovo; [4]Department of Medicine, University of Pristina, Pristina, Kosovo; [5]Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York, New York, USA; [6]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, USA

Address correspondence to Pam Factor-Litvak, Department of Epidemiology, Mailman School of Public Health, 722 West 168th Street, 16th floor, New York, NY 10032. Telephone (212) 305-7851. E-mail: prf1@columbia.edu

Running title: Blood lead and thyroid function during pregnancy

Acknowledgments: Supported by NIEHS grants R01ES011142, R01ES03460 and P30ES09089.

Competing financial interests: No competing financial interests.

Abstract

Background: Although maternal hypothyroidism increases the risk of adverse neonatal and obstetric outcomes as well as lower IQ in children, the environmental determinants of maternal thyroid dysfunction have yet to be fully explored.

Objectives: We aimed to examine associations between mid-pregnancy blood lead (BPb) and concomitant measures of thyroid function among participants in the Yugoslavia Prospective Study of Environmental Lead Exposure.

Methods: As part of a population-based prospective study of two towns in Kosovo, one with high levels of environmental lead and one with low, women were recruited during the second trimester of pregnancy, at which time blood samples and questionnaire data were collected. We measured concentrations of BPb, free thyroxine (FT4), thyroid stimulating hormone (TSH), and thyroid peroxidase antibodies (TPO Ab) in archived serum samples.

Results: Compared to women from the unexposed town, women from the exposed town had lower mean FT4 (0.91 ± 0.17 vs. 1.03 ± 0.16 ng/dL), higher mean TPO Ab (15.45 ± 33.08 vs. 5.12 ± 6.38 IU/mL) and higher mean BPb (20.00 ± 6.99 vs. 5.57 ± 2.01 μg/dL). No differences in TSH levels were found. After adjustment for potential confounders, for each natural log unit increase in BPb, FT4 decreased by 0.074 ng/dL, 95% confidence interval (CI): -0.10, -0.046 ng/dL, and the odds ratio for testing positive to TPO Ab was 2.41, 95% CI: 1.53, 3.82. We found no association between BPb and TSH.

Conclusions: Prolonged lead exposure may contribute to maternal thyroid dysfunction by stimulating autoimmunity to the thyroid gland.

Introduction

The adverse effects of early childhood exposure to high levels of environmental lead are well established (Needleman and Landrigan 1991). In some but not all studies, higher prenatal lead exposure (blood lead (BPb) 10-20 μg/dL) is associated with a wide range of adverse pregnancy outcomes (Bellinger 2005), including shorter gestational lengths (Cantonwine et al. 2010); reduced birth weight (Bellinger et al. 1991; Gonzalez-Cossio et al. 1997), birth length, and head circumference (Hernandez-Avila et al. 2002); deficits in infant mental development (Gomaa et al. 2002); and decreased child IQ (Schnaas et al. 2006; Wasserman et al. 2000; Wasserman et al. 1998). Elevated prenatal exposure to lead may be associated with adult-onset psychiatric disorders such as schizophrenia (Opler et al. 2004; Opler et al. 2008). While mean BPb levels in the U.S. declined precipitously following the removal of lead from gasoline and most paint in the mid-1970s, the greatest decline in IQ among children occurs at the lowest levels of exposure (Lanphear et al. 2005), indicating that there may be no safe level of lead exposure (Bellinger 2008). In large areas of the world, where the mining, smelting, and refining of lead and the manufacture and recycling of lead-containing products such as batteries, computers, and solar panels are not closely monitored, lead poisoning is still a serious health concern for children. A recent episode of acute lead poisoning related to artisanal gold processing in a village in northwestern Nigeria that killed 25% of the under-five population emphasizes the hazard that lead continues to pose in many places around the world (Dooyema et al. 2012).

A recent report on a U.S. national sample of more than half a million pregnant women found that 15.5% of those screened tested positive for either clinical (elevated thyroid stimulating hormone (TSH) and reduced free thyroxine (FT4)) or subclinical (elevated TSH and normal FT4) hypothyroidism, far higher than previous estimates (Blatt et al. 2012). Prevalences in other parts

of the world, especially developing countries where iodine deficiency is still a public health problem, have been found to be even greater (Mbah et al. 2011). Despite its high prevalence and negative outcomes, little is known about the predictors of clinical and subclinical gestational hypothyroidism aside from iodine deficiency. Maternal iodine intake must increase by 50% to fuel the increase in thyroid hormone production during pregnancy (Stagnaro-Green and Pearce 2013), and even mild to moderate first trimester gestational iodine deficiency can lead to decrements in verbal IQ and reading ability in school-age children (Bath et al. 2013). Other variables reported to be associated with gestational hypothyroidism include larger maternal thyroid size, higher gravidity, higher pre-pregnancy body mass index (BMI), and increased fetal gestational age (Boas, Forman et al. 2009; Mbah et al. 2011). Animal studies and studies of acute human exposure indicate that numerous chemicals interfere with thyroid hormone regulation and function (Hartoft-Nielsen et al. 2011; Pearce and Braverman 2009; Boas, Main et al. 2009). However, few studies assess the associations between persistent lower-dose environmental exposures on thyroid function, and even fewer consider these in pregnant women.

The deleterious effect of gestational hypothyroidism on fetal brain development is well documented (Escobar et al. 2004). Additionally, the presence of maternal thyroid peroxidase antibodies (TPO Ab) during late gestation has been associated with reduced child IQ at age 5 even when controlling for postpartum thyroid dysfunction and maternal depression (Pop et al. 1995). While untreated maternal thyroid dysfunction has been associated with a reduction of up to seven IQ points in school-aged children (Haddow et al. 1999), there have been inconsistent results of studies in which mothers were treated. Man and Serunian found lower psychological scores among 7-year-old children of mothers with inadequately treated prenatal hypothyroxinemia compared to the children of adequately treated and euthyroid women (Man

and Serunian 1976), while Lazarus et al. recently reported comparable mean IQ scores at age 3 among children whose mothers were randomized to be screened and, if necessary, treated for gestational hypothyroidism compared to children whose mothers were not screened or treated (Lazarus et al. 2012).

Building on previous occupational studies and on studies in small general population samples (Bledsoe et al. 2011; Dundar et al. 2006; Mbah et al. 2011; Tuppurainen et al. 1988), we hypothesized that maternal BPb might be associated with reduced thyroid function via one of three possible pathways. One potential mechanism involves iodine, adequate levels of which are essential for normal thyroid function. More than half a century ago, Slingerland demonstrated impaired uptake of iodine by fresh sheep thyroid tissue exposed to lead nitrate in solution (Slingerland 1955). Sandstead et al's subsequent study of individuals exposed to lead either occupationally or through ingestion of tainted whiskey demonstrated a similar association in humans (Sandstead et al. 1969). The second pathway involves disruption of the release of transthyretin (TTR) into the cerebrospinal fluid, preventing the transport of FT4 to the brain. Lead is sequestered in the choroid plexus, the region of the brain where brain-specific TTR is produced. In both rodent and human studies, Zheng et al have shown BPb to be inversely associated with both TTR and FT4 (Zheng et al. 2001; Zheng et al. 1996). In both of these scenarios, we would expect to see elevated maternal mid-pregnancy TSH, reduced mid-pregnancy FT4, and no discernible TPO Ab. Finally, we speculated that lead may affect thyroid function by triggering autoimmune thyroiditis (AT). In the case of such a direct assault on the thyroid gland, we would expect to see elevated TPO Ab and depressed FT4, but no effect on TSH.

In order to test these three potential pathways, we examined the associations between BPb and measures of FT4, TSH and TPO Ab in data collected during the Yugoslavia Prospective Study of Lead Exposure, Pregnancy Outcomes and Child Development (Graziano et al. 1990). To our knowledge, this is the first study to explore the relationship between lead exposure and thyroid function in a sample of pregnant women.

Methods

Study population

Between May 1985 and December 1986, women in their second trimester of pregnancy were invited to participate in a study of pregnancy outcomes at their first prenatal visit to government clinics located at the centers of two towns in Kosovo. Details of the study design have been published previously (Factor-Litvak et al. 1991; Factor-Litvak et al. 1999; Graziano et al. 1990; Wasserman et al. 1998). A total of 1502 women were recruited: 602 from Mitrovica, a town with a lead smelter, refinery, and battery plant in which high BPb concentrations had been reported in both adults and children (Popovac et al. 1982); and 900 from Pristina, 25 miles to the south, where the primary source of environmental lead was gasoline, as lead-based paint has been banned in Yugoslavia since 1922. Complete delivery data were available on 1008 mother-infant pairs. Inclusion criteria for continuing in the study were: giving birth to a single child between 18 and 44 weeks gestation who was free of major central nervous system defects or chromosomal abnormalities, and living within 10 kilometers of the clinic. The 394 infants from Mitrovica with available cord blood BPb measures were then divided into three groups: < 15 μg/dL, 15-20 μg/dL, and > 20 μg/dL. Two groups of infants from Pristina were selected for follow-up: one group frequency-matched on BPb concentration to the group from Mitrovica with BPb < 15 μg/dL, and a second group matched on maternal and paternal education to the group from

Mitrovica with BPb > 20 μg/dL. Of the resulting 711 infants invited to continue in the study, the parents of 541 consented. The sample for the present analyses included 291 women enrolled in mid-pregnancy who had adequate serum in storage to measure FT4, TSH, and TPO Ab levels at the time of a follow-up study of prenatal thyroid function, lead, and child growth at age 7 (Lamb et al. 2008), and did not display overt hypothyroidism, defined as TSH > 2.5 μIU/mL and FT4 < 0.7 ng/dL, the latter cutoff value representing the lowest 5th percentile of the sample population (Stagnaro-Green et al. 2011) (Figure 1). This study was approved by the Columbia University IRB and by the "Komitetietik," an NIH-registered IRB at the University of Pristina, Kosovo. All women gave written informed consent prior to the study.

Data collection

At their first prenatal visit, pregnant women enrolled in this study were interviewed by trained bilingual (Serbo-Croatian and Albanian) interviewers, who collected data on socio-demographic criteria, pregnancy and health history, and lifestyle variables. Fetal gestational age at interview ranged from 9 to 28 weeks, with a mean of 18 completed weeks. The nurses measured the women's height and weight and obtained venous blood samples, which were refrigerated on site and transported on wet ice to Columbia University. After transport to Columbia, blood was stored at -20°C for several months until analyzed for lead, hemoglobin, erythrocyte protoporphyrin, and serum ferritin; samples with evidence of hemolysis were excluded. The remaining blood and serum was stored at -20°C and the thyroid measures were analyzed approximately 15 years after collection. Pilot data indicated that the values of FT4, TSH, and TPO Ab were in the range expected for women during mid-pregnancy.

Blood lead

Mid-pregnancy maternal serum samples were assayed for BPb according to methods described previously (Factor-Litvak et al. 1991). The Columbia laboratory participates in the Centers for Disease Control and Prevention (CDC) quality control program for BPb analyses and is certified by the Occupational Safety and Health Administration; during the course of the study, the intraclass correlation coefficient for agreement with CDC values for BPb was 0.95. All samples had BPb levels above the detection limit of 0.1 μg/dL.

Maternal thyroid measures

Maternal thyroid function during pregnancy was assessed using FT4, TSH, and TPO Ab, all of which have been shown to resist deterioration during freezing, storage, and thawing (Mannisto et al. 2007). FT4 and TPO Ab were measured by a radioimmunoassay procedure and TSH was measured using an IRMA procedure (all by ICN Biomedicals, Costa Mesa, CA). According to the technical specifications of the assay, TPO Ab was characterized as slightly elevated if TPO Ab levels were ≥ 10 IU/mL and < 20 IU/mL, moderately elevated if ≥ 20 IU/mL and < 100 IU/mL, and highly elevated if ≥ 100 IU/mL. For the purpose of this study, all cases with slightly, moderate, or highly elevated TPO Ab levels were considered positive. Euthyroid women with elevated TPO Ab levels were considered at risk of hypothyroidism (Stagnaro-Green et al. 2011).

Statistical analyses

We natural log transformed BPb, TSH, and TPO Ab to meet assumptions of the statistical models and to reduce the influence of extreme values. Preliminary analyses evaluated potential confounding variables including maternal age, fetal gestational age at blood sample [because measures of thyroid hormone vary during the course of pregnancy (Glinoer 2000)], town (to account for unspecified geographic factors that might influence thyroid hormone level in

pregnancy), anthropometric measures (maternal height, pre-pregnancy weight, and BMI), hemoglobin (Hgb), lifestyle characteristics (smoking, alcohol use, and coffee consumption), and socio-demographics (ethnicity, maternal education, parity, ratio of rooms to number of adults in household, and home ownership). Specifically, we used analysis of variance (ANOVA) to compare means of continuous outcome variables according to levels of categorical predictor variables. We calculated Spearman correlation coefficients to assess bivariate associations between continuous predictor and outcome variables. Multiple linear regression analysis was used to estimate covariate-adjusted associations between BPb and the continuous outcome measures of thyroid function and logistic regression analysis to assess the relationship between BPb and the binary outcome measure TPO Ab. Outcome-specific covariates were identified in preliminary analyses as variables associated with BPb and the specific outcome at $p < 0.2$. We also identified covariates as those found in previous studies to be associated with the outcome (Boas, Forman et al. 2009; Mbah et al. 2011). We graphically examined the relationships between BPb and outcome measures and additionally ran our regression models substituting town for BPb as the main predictor variable. In sensitivity analyses restricted to Albanian women, associations between BPb and thyroid outcome measures were unchanged, indicating that ethnicity was not a major confounder (data not shown). We also found no difference when we included a quadratic term for fetal gestational age in our models and concluded that our results were not affected by a non-linear association between gestational age and thyroid measures (data not shown). All statistical tests were two-tailed, with a significance level of 0.05. Data were analyzed using SAS® 9.2 statistical software (SAS Institute, Inc., Cary, NC).

Results

At recruitment, the 291 subjects used in this analysis were similar to the 420 members of the cohort who did not meet the inclusion criteria in terms of age, education, number of prior live births, mid-pregnancy BPb and Hgb levels, and fetal gestation age at mid-pregnancy blood draw. Women from the two towns were comparable on all of these measures except for BPb levels. The only notable difference between those included and not included is that the distribution of ethnicities between the two towns, which had been comparable at the time of recruitment, was no longer after loss to follow-up over the subsequent 7 years, likely due to migration during the mounting ethnic tensions in the late 1980s and early 1990s. In Pristina, the proportion of Albanian participants increased (from 59.0% in the original sample to 70.8% after loss to follow-up), the proportion of Serbian participants decreased (from 28.5% to 22.5%), and the proportion of other ethnicities decreased (from 12.5% to 6.8%), while in Mitrovica, the distribution did not change (53.4% vs. 54.9% Albainan, 28.65% vs. 27.1% Serbian, 18.0% vs. 18.1% other). In Mitrovica, those included had slightly higher mid-pregnancy BPb compared to those lost to follow-up (20.0 vs. 18.5 μg/dL), and among those included in the study, women in Mitrovica had slightly fewer prior live births compared to those in Pristina (mean 1.4 vs. 1.7), but neither of these differences reached statistical significance (Table 1).

Among the participants, we found highly significant differences between the two towns in both FT4 and TPO Ab (p-value < 0.0001), but not in TSH (Table 2). Women from Mitrovica, who were more highly exposed to lead (mean BPb 20.00 vs. 5.57 μg/dL), had lower mean FT4 (0.91 vs. 1.03 ng/dL) and higher mean TPO Ab (15.45 vs. 5.12 IU/mL), both indicative of higher risk of gestational hypothyroidism. Out of the 291 women in our sample, 24 (8.25%) had FT4 levels below 0.7 ng/dL, the commonly-used cutoff for hypothyroidism (Blatt et al. 2012), and 57

(19.59%) tested positive for TPO Ab (≥ 10 IU/mL). Among those with positive TPO Ab, 38 (66.67%) had slightly elevated levels (≥ 10 IU/mL and < 20 IU/mL), 13 (22.81%) had moderately elevated levels (≥ 20 IU/mL and < 100 IU/mL), and 6 (10.53%) had highly elevated levels (≥ 100 IU/mL). Most strikingly, the prevalence of elevated TPO Ab (≥ 10 IU/mL) was nearly five times greater among women in Mitrovica compared to women in Pristina (32.64% vs. 6.80%) (data not shown).

In bivariate analyses (Supplemental Material, Table S1), BPbs were significantly associated with town, ethnicity, maternal height, and fetal gestational age at blood draw. FT4 was significantly associated with ethnicity, maternal education, pre-pregnancy BMI, and crowded living conditions. TPO Ab was significantly associated with smoking status. As expected, there was an inverse association between BMI and FT4. We also found that Albanians had higher mean FT4 than Serbians (0.99 ± 0.17 vs. 0.89 ± 0.16 ng/dL, respectively), that those with no education had higher mean FT4 than those with any, and that there was a positive association between FT4 and adults per room. These results reflect associations between height and ethnicity (p-value < 0.01), between ethnicity and education (p-value < 0.0001), and between ethnicity and adults per room (p-value < 0.001). In contrast to published findings of a protective relationship between smoking and thyroid autoimmunity (Belin et al. 2004; Effraimidis et al. 2009; Escobar et al. 2004), in our cohort smoking was associated with a higher mean TPO Ab level. TSH was not significantly associated with any of the characteristics we selected as potential covariates.

Scatter plots between BPb and the three outcome variables, adjusted for potential confounders (Figure 2), suggest an inverse relationship between BPb and FT4 and a direct relationship between BPb and TPO Ab, but no association between BPb and TSH.

BPb was negatively associated with FT4 and positively associated with TPO Ab in both covariate adjusted and unadjusted models (p-values < 0.0001) (Table 3); no association was found between BPb and TSH. Controlling for potential confounders, for each log unit increase in BPb, FT4 decreased by 0.074 ng/dL (95% CI: -0.10, -0.046 ng/dL). Using logistic regression to adjust for ethnicity, fetal gestational age, maternal age, and adults per room (a proxy measure for socioeconomic status), we found the estimated odds of testing positive for TPO Ab to be 2.41 times greater for every log-unit increase in mid-pregnancy BPb (95% CI: 1.53, 3.82).

Discussion

The current study, an analysis of mid-pregnancy BPb compared to mid-pregnancy FT4, TSH, and TPO Ab levels, yielded a highly significant negative association between BPb and FT4 and a highly significant positive association between BPb and TPO Ab without any significant association between BPb and TSH. These results indicate that lead exposure may be a factor in reduced thyroid function, which has been suggested to increase the risk of poor obstetric outcomes (Casey et al. 2005, van den Boogaard et al. 2011, Ajmani et al. 2014) and lower IQ in children (Man and Serunian 1976, Pop et al. 1995, Haddow et al. 1999). These results suggest the plausibility of the latter of the three potential pathways by which we hypothesized that BPb might be associated with reduced thyroid function: via reduced uptake of iodine by thyroid tissue, via disruption of the release of TTR from the choroid plexus, and via the triggering of an autoimmune response to the maternal thyroid gland.

The Yugoslavia Prospective Study of Environmental Lead Exposure, Pregnancy Outcomes and Child Development (Graziano et al. 1990), is one of several longitudinal cohort studies designed to explore the effects of long-term lead exposure on pregnant women and their offspring (Bellinger et al. 1987; Canfield et al. 2003; Dietrich et al. 1987; Ernhart et al. 1989; McMichael

et al. 1988; Schnaas et al. 2006). Two towns were chosen with relatively low and high environmental lead. The mean mid-pregnancy BPb among women in Mitrovica, an industrial town with a lead smelter, refinery, and battery plant, was nearly four times higher than among women in Pristina, the capital of Kosovo (20.01 vs 5.57 μg/dL). By contrast, according to the most comparable U.S. data available, from Phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III), mean blood lead level among adults age 20-49 measured between 1988 and 1991 was 2.6 μg/dL (Brody et al. 1994).

Although generally considered to be a marker of recent Pb exposure, BPb reflects both exogenous (environmental) and endogenous (bone, tissue) Pb sources, and may also be viewed as a marker of cumulative Pb exposure (Factor-Litvak et al. 1999). Indeed, because bone is remodeled during pregnancy (Hertz-Picciotto et al. 2000), BPb measured during pregnancy reflects both current and more chronic exposures. To further explore the relationship between long-term Pb exposure and our outcome measures, we reran our regression models using town as the main predictor variable. We considered town to be a good proxy for long-term Pb exposure because it was strongly associated with BPb in our cohort and because maternal blood samples were taken prior to the breakup of Yugoslavia, during a time when the residential population was relatively stable. In Mitrovica, where women were more highly exposed to lead, mean FT4 levels were lower (0.91 vs. 1.03 ng/dL) and TPO Ab levels were higher (15.45 vs. 5.12 IU/mL) than in Pristina. Perhaps most striking, the large difference in mean TPO Ab between the two towns lends credence to our hypothesis that that the relationship between long-term lead exposure and gestational thyroid dysfunction might be through the autoimmune pathway.

While no previous studies have examined associations between lead exposure and AT or elevated TPO Ab levels, studies have examined associations between other environmental

exposures and the disease (Brent 2010). AT is generally acknowledged to be multifactorial, with both genetic and environmental components. Iodine has been shown to be a trigger of overt hypothyroidism in studies of patients with asymptomatic AT who were administered excessive dietary iodine (Braverman et al. 1971; Tajiri et al. 1986). Similarly, those with preexisting AT are more likely to develop hypothyroidism than those without TPO Ab when given lithium (Bocchetta and Loviselli 2006). Selenium deficiency (Duntas 2010) and vitamin B_{12} deficiency (Lahner et al. 2008) have also been implicated in AT. In small observational studies, elevated TPO Ab levels have been positively associated with exposure to organochlorines (Langer et al. 2008), polychlorinated biphenyls (Langer et al. 2007), and polyhalogenated biphenyls (Bahn et al. 1980). Studies using genetically predisposed mice have also shown bromine and bacterial lipopolysaccharides to be triggers of AT (Burek and Talor 2009).

Lead is known to affect the immune system, but in ways that are still not clearly understood (Dietert and Piepenbrink 2006). In vitro and in vivo studies in mice have suggested that lead initially skews T-lymphocyte response toward the Th2 pathway (McCabe and Lawrence 1991, Heo et al. 1998), increasing the risk of asthma and atopy, although a subsequent shift back to the Th1 pathway, observed in different mouse study, could result in a predisposition to autoimmunity (Goebel et al. 2000). In a study of mice genetically predisposed to systemic lupus erythematosus, lead exposure triggered onset of the disease (Hudson et al. 2003). Lead has also been shown to stimulate production of autoantibodies against neural proteins in both rodent models and human occupational studies (El-Fawal et al. 1999; Waterman et al. 1994).

There are several limitations to our study of lead exposure and gestational thyroid dysfunction. The sample size, while large enough to produce robust findings when data from the two towns were combined, was not large enough to support statistically significant findings when analyses

were stratified by town, even though the parameter estimates were similar in the combined and stratified models (Supplemental Material, Table S2). While the original study sample was selected to achieve broad representation across lead exposure levels and socio-economic status, the current study relied on the subsample for which we had mid-pregnancy thyroid measures. There is no reason to believe that such loss to follow-up would bias the biological relationships between BPb and thyroid outcome measures. Although it is possible that hormones may have degraded between the time between serum collection and analysis, we do not believe this was a major concern, as mean TSH levels are comparable to what would be expected in women during mid-pregnancy. Because thyroid binding globulin may impede the reliability of FT4 assays, it is preferable to use circulating total thyroxine as a measure of thyroid gland activity in pregnant women, as thyroid binding globulin concentrations are elevated during pregnancy (Stagnaro-Green et al. 2011). Unfortunately, we did not have direct measures of total thyroxine and or thyroid binding globulin in our data. Finally, our data did not include mid-pregnancy urinary iodine measures, preventing us from definitively ruling out the possibility that lead causes gestational thyroid dysfunction by impairing uptake of iodine by the thyroid gland.

Conclusions

This study contributes unique information to our understanding of lead and gestational thyroid dysfunction. Our findings suggest that long-term lead exposure increases the risk of elevated TPO Ab during pregnancy, adding to the growing literature on the environmental influences on AT. While the results of this study are limited to pregnant women, future studies might extend them to examine the effect of prenatal lead exposure on TPO Ab levels in children as well as on the development of postnatal hypothyroidism among the mothers.

References

Ajmani SN, Aggarwal D, Bhatia P, Sharma M, Sarabhai V, Paul M. Prevalence of overt and subclinical thyroid dysfunction among pregnant women and its effect on maternal and fetal outcome. 2014. J Obstet Gynaecol India. 64(2):105-110.

Bahn AK, Mills JL, Snyder PJ, Gann PH, Houten L, Bialik O, et al. 1980. Hypothyroidism in workers exposed to polybrominated biphenyls. N Engl J Med 302:31-33.

Bath S, Steer C, Golding J, Emmett P, Rayman M. 2013. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet 382(9889):331-337.

Belin R, Astor B, Powe N, Ladenson P. 2004. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 89(12):6077-6086.

Bellinger D. 2005. Teratogen update: Lead and pregnancy. Birth Defects Res A Clin Mol Teratol 73:409-420.

Bellinger D. 2008. Very low lead exposures and children's neurodevelopment. Curr Opin Pediatr 20(2):172-177.

Bellinger D, Leviton A, Rabinowitz M, Allred E, Needleman H, Schoenbaum S. 1991. Weight gain and maturity in fetuses exposed to low levels of lead. Environ Res 54(2):151-158.

Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. 1987. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N Engl J Med 316(17):1037-1043.

Blatt A, Nakamoto J, Kaufman H. 2012. National status of testing for hypothyroidism during pregnancy and postpartum. J Clin Endocrinol Metab 97(3):777-784.

Bledsoe M, Pinkerton L, Silver S, Deddens J, Biagini R. 2011. Thyroxine and free thyroxine levels in workers occupationally exposed to inorganic lead. Environ Health Insights 5:55-61.

Boas M, Forman J, Juul A, Feldt-Rasmussen U, Skakkebaek N, Hilsted L, et al. 2009. Narrow intra-individual variation of maternal thyroid function in pregnancy based on a longitudinal study on 132 women. Eur J Endocrinol 161:903-910.

Boas M, Main KM, Feldt-Rasmussen U. 2009. Environmental chemicals and thyroid function: an update. Curr Opin Endocrinol Diabetes Obes 16(5):385-391.

Bocchetta A, Loviselli A. 2006. Lithium treatment and thyroid abnormalities. Clin Pract Epidemiol Ment Health 2(23). doi:10.1186/1745-0179-2-23.

Braverman LE, Ingbar SH, Vagenakis AG, Adams L, Maloof F. 1971. Enhanced susceptibility to iodine myxedema in patients with Hashimoto's disease. J Clin Endocrinol Metab 32:515-521.

Brent G. 2010. Environmental exposures and autoimmune thyroid disease. Thyroid 20(7):755-761.

Brody D, Pirkle J, Kramer R, Flegal K, Matte T, Gunter E, et al. 1994. Blood lead levels in the US Population: Phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1991). JAMA 272(4):277-283.

Burek CL, Talor MV. 2009. Environmental triggers of autoimmune thyroiditis. J Autoimmun 33(3-4):183-189.

Canfield R, Henderson C, Cory-Slechta D, Cox C, Jusko T, Lanphear B. 2003. Intellectual impairment in children with blood lead concentrations below 10 μg per deciliter. N Engl J Med 348(16):1517-1526.

Cantonwine D, Hu H, Sanchez B, Lamadrid-Figueroa H, Smith D, Ettinger A, et al. 2010. Critical windows of fetal lead exposure: Adverse impacts on length of gestation and risk of premature delivery. J Occup Environ Med 52(11):1106-1111.

Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Levenko KJ, Cunningham FG. 2005. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol 105(2):239-245.

Dietert RR, Piepenbrink MS. 2006. Lead and immune function. Crit Rev Toxicol 36:359-385.

Dietrich K, Krafft K, Bornschein R, Hammond P, Berger O, Succop P, et al. 1987. Low-level fetal lead exposure effect on neurobehavioral development in early infancy. Pediatrics 80(5):721-730.

Dooyema C, Neri A, Lo Y-C, Durant J, Dargan P, Swarthout T, et al. 2012. Outbreak of fatal childhood lead poisoning related to artisanal gold mining in northwestern Nigeria, 2010. Environ Health Perspect 120(4):601-607.

Dundar B, Oktem F, Arslan M, Delibas N, Baykal B, Arslan C, et al. 2006. The effect of long-term low-dose lead exposure on thyroid function in adolescents. Environ Res 101:140-145.

Duntas LH. 2010. Selenium and the thyroid: A close-knit connection. J Clin Endocrinol Metab 95(12):5180-5188.

Effraimidis G, Tijssen J, Wiersinga W. 2009. Discontinuation of smoking increases the risk for developing thyroid peroxidase antibodies and/or thyroglobulin antibodies: a prospective study. J Clin Endocrinol Metab 94(4):1324-1328.

El-Fawal HA, Waterman SJ, Feo AD, Shamy MY. 1999. Neuroimmunotoxicology: Humoral assessment of neurotoxicity and autoimmune mechanisms. Environ Health Perspect 107(Suppl 5):767-775.

Ernhart C, Morrow-Tlucak M, Wolf A, Super D, Drotar D. 1989. Low level lead exposure in the prenatal and early preschool periods: intelligence prior to school entry. Neurotoxicol Teratol 11(2):161-170.

Escobar Gd, Obregon M, Rey Fd. 2004. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab 18(2):225-248.

Factor-Litvak P, Graziano J, Kline J, Popovac D, Mehmeti A, Ahmedi G, et al. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. Int J Epidemiol 20:722-728.

Factor-Litvak P, Wasserman G, Kline J, Graziano J. 1999. The Yugoslavia Prospective Study of Environmental Lead Exposure. Environ Health Perspect 107(1):9-15.

Glinoer D. 2000. Thyroid disease during pregnancy. In: Werner and Ingbar's 'The Thyroid: A Fundamental and Clinical Test', (Braverman L, Utiger R, eds). Philadelphia, PA:Lippincott Williams & Wilkins, 1013-1027.

Goebel C, Flohe SB, Kirchhoff K, Herder C, Kolb H. 2000. Orally administered lead chloride induces bias of mucosal immunity. Cytokine 12(9):1414-1418.

Gomaa A, Hu H, Bellinger D, Schwartz J, Tsaih S, Gonzalez-Cossio T, et al. 2002. Maternal bone lead as an independent risk factor for fetal neurotoxicity: A prospective study. Pediatrics 110(1 Part 1):110-118.

Gonzalez-Cossio T, Peterson K, Sanin L, Fishbein E, Palazuelos E, Aro A, et al. 1997. Decrease in birth weight in relation to maternal bone-lead burden. Pediatrics 100(5):856-862.

Graziano J, Popovac D, Factor-Litvak P, Shrout P, Kline J, Murphy M, et al. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environ Health Perspect 89:95-100.

Haddow J, Palomaki G, Allan W, Williams J, Knight G, Gagnon J, et al. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341(8):549-555.

Hartoft-Nielsen M, Boas M, Bliddal S, Rasmussen A, Main K, Feldt-Rasmussen U. 2011. Do thyroid disrupting chemicals influence foetal development during pregnancy? J Thyroid Res doi:10.4061/2011/342189.

Heo Y, Lee WT, Lawrence DA. 1998. Differential effects of lead and cAMP on development and activities of Th1- and Th2-lymphocytes. Toxicol Sci 43(2):172-185.

Hernandez-Avila M, Peterson K, Gonzalez-Cossio T, Sanin L, Aro A, Schnaas L, et al. 2002. Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants. Arch Environ Health 57(5):482-488.

Hertz-Picciotto I, Schramm M, Watt-Morse M, Chantala K, Anderson J, Osterloh J. 2000. Patterns and determinants of blood lead during pregnancy. Am J Epidemiol 152(9):829-837.

Hudson CA, Cao L, Kasten-Jolly J, Kirkwood JN, Lawrence DA. 2003. Susceptibility of lupus-prone NZM mouse strains to lead exacerbation of systemic lupus erythematosus symptoms. J Toxicol Environ Health 66(10):895-918.

Lahner E, Centanni M, Agnello G, Gargano L, Vannella L, Iannoni C, et al. 2008. Occurrence and risk factors for autoimmune thyroid disease in patients with atrophic body gastritis. Am J Med 121(2):136-141.

Lamb MR, Janevic T, Liu X, Cooper T, Kline J, Factor-Litvak P. 2008. Environmental lead exposure, maternal thyroid function, and childhood growth. Environ Res 106(2):195-202.

Langer P, Kocan A, Tajtakova M, Koska J, Radikova Z, Ksinantova L, et al. 2008. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: Outcome of transgenerational transmission? Chemosphere 73(7):1145-1150.

Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, et al. 2007. Fish from industrially poluted freshwater as the main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere 67(9):S379-385.

Lanphear B, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger D, et al. 2005. Low-level environmental lead exposure and children's intellectual function: An international pooled analysis. Environ Health Perspect 113(7):894-899.

Lazarus J, Bestwick J, Channon S, Paradice R, Maina A, Rees R, et al. 2012. Antenatal thyroid screening and childhood cognitive function. New Engl J Med 366(6):493-501.

Man E, Serunian S. 1976. Thyroid function in human pregnancy. IX. Development or retardation of 7-year-old progeny of hypothyroxinemic women. Am J Obstet Gynecol 125(7):949.

Mannisto T, Surcel H, Bloigu A, Ruokonen A, Hartikainen A, Jarvelin M, et al. 2007. The effect of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid autoantibodies: implications for analyzing samples stored in serum banks. Clin Chem 53(11):1986-1987.

Mbah A, Ejim E, Onodugo O, Ezugwu F, Eze M, Nkwo P, et al. 2011. Two logistic models for the prediction of hypothyroidism in pregnancy. BMC Res Notes 4:205-214.

McCabe MJ, Lawrence DA. Lead, a major environmental pollutant, is immunomodulatory by its differential effects on CD4+ T cell subsets. 1991. Toxicol Appl Pharmacol 111(1):13-23.

McMichael A, Baghurst P, Wigg N, Vimpani G, Robertson E, Roberts R. 1988. Port Pirie Cohort Study: environmental exposure to lead and children's abilities at the age of four years. N Engl J Med 319(8):468-475.

Needleman H, Landrigan P. 1991. The health effects of low level exposure to lead. Annu Rev Public Health 2:277-298.

Opler M, Brown A, Graziano J, Desai M, Zheng W, Schaefer C, et al. 2004. Prenatal lead exposure, delta-aminolevulinic acid, and schizophrenia. Environ Health Perspect 112(5):548-552.

Opler M, Buka S, Groeger J, McKeague I, Wei C, Factor-Litvak P, et al. 2008. Prenatal exposure to lead, delta amniolevulinic acid, and schizophrenia: Further evidence. Environ Health Perspect 116(11):1586-1590.

Pearce EN, Braverman, LE. 2009. Environmental pollutants and the thyroid. Best Pract Res Clin Endocrinol Metab 23(6):801-813.

Pop V, deVries E, vanBaar A, Waelkens J, deRooy H, Horsten M, et al. 1995. Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development? J Clin Endocrinol Metab 80(12):3561-3566.

Popovac D, Graziano J, Seaman C, Kaul B, Colakovic B, Popovac R, et al. 1982. Elevated blood lead in a population near a lead smelter in Kosovo, Yugoslavia. Arch Environ Health 37:19-23.

Sandstead H, Stant E, Brill A, Arias L, Terry R. 1969. Lead intoxication and the thyroid. Arch Intern Med 123:632-635.

Schnaas L, Rothenberg S, Flores F, Martinez S, Hernandez C, Osorio E, et al. 2006. Reduced intellectual development in children with prenatal lead exposure. Environ Health Perspect 114(5):791-797.

Slingerland D. 1955. The influence of various factors on the uptake of iodine by the thyroid. J Clin Endocrinol Metab 15:131-141.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. 2011. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 21(10):1081-1125.

Stagnaro-Green A, Pearce EN. 2013. Iodine and pregnancy: a call to action. Lancet 382(9889):292-293.

Tajiri J, Higashi K, Morita M, Umeda T, Sato T. 1986. Studies of hypothyroidism in patients with high iodine intake. J Clin Endocrinol Metab 63:412-417.

Tuppurainen M, Wagar G, Kurppa K, Sakari W, Wambugu A, Froseth B, et al. 1988. Thyroid function as assessed by routine laboratory tests of workers with long-term lead exposure. Scand J Work Environ Health 14:175-180.

van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, Bisschop PH. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. 2011. Hum Reprod Update. 17(5):605-619.

Wasserman G, Liu X, Popovac D, Factor-Litvak P, Kline J, Waternaux C, et al. 2000. The Yugoslavia Prospective Lead Study: contributions of prenatal and postnatal lead exposure to early intelligence. Neurotoxicol Teratol 22(6):811-818.

Wasserman G, Staghezza-Jaramillo B, Shrout P, Popovac D, Graziano J. 1998. The effect of lead exposure on behavior problems in preschool children. Am J Public Health 88:481-486.

Waterman SJ, El-Fawal HA, Snyder CA. 1994. Lead alters the immunogenicity of two neural proteins: A potential mechanism for the progression of lead-induced neurotoxicity. Environ Health Perspect 102(12):1052-1056.

Zheng W, Lu Y, Lu G, Zhao Q, Cheung O, Blaner W. 2001. Transthyretin, thyroxine, and retinol-binding protein in human cerebrospinal fluid: Effect of lead exposure. ToxicolSci 61(1):107-114.

Zheng W, Shen H, Blaner W, Zhao Q, Ren X, Graziano J. 1996. Chronic lead exposure alters transthyretin concentration in rat cerebrospinal fluid: The role of the choroid plexus. Toxicol Appl Pharmacol 139(2):445-450.

Table 1. Participants compared to members of the Yugoslavia Prospective Study of Environmental Lead Exposure cohort lost to follow-up by child age 7.

	Included, Pristina (n=147)	Included, Mitrovica (n=144)	Range	p- value	Lost to follow-up, Pristina (n=165)	Lost to follow-up, Mitrovica (n=255)	Range	p-value
Maternal age (years)	26.6 ± 4.7	26.7 ± 5.2	16.1-41.7	0.87	26.7 ± 4.5	26.1 ± 4.7	15.1-46.0	0.19
Maternal education (years)	9.2 ± 3.9	9.3 ± 3.8	0-17	0.87	9.8 ± 3.8	9.4 ± 4.0[a]	0-17	0.29
# Prior live births	1.7 ± 1.7	1.4 ± 1.6	0-9	0.099	1.5 ± 1.4	1.4 ± 1.5	0-9	0.53
Mid-pregnancy BPb (µg/dL)	5.6 ± 2.0	20.0 ± 7.0	1.6-41.3	<0.0001	5.8 ± 2.1[b]	18.5 ± 7.9[c]	1.7-43.4	<0.0001
Gestational age at birth (days)	276.2 ± 18.6	274.3 ± 18.1[d]	195-333	0.38	274.6 ± 18.7[e]	274.3 ± 18.1[f]	164-308	0.87
Gestational age at blood draw (days)	132.7 ± 26.3	120.7 ± 26.8	61-192	0.0001	134.9 ± 30.9[g]	119.2 ± 25.8[h]	47-220	<0.0001
Maternal ethnicity				0.0041				0.61
Albanian	104 (70.8)	79 (54.9)			80 (48.5)	134 (52.6)		
Serbian	33 (22.5)	39 (27.1)			56 (33.9)	75 (29.4)		
Other	10 (6.8)	26 (18.1)			29 (17.6)	46 (18.0)		
Smoking during pregnancy	42 (28.6)	34 (23.6)		0.34	50 (31.9)[e]	67 (28.9)[f]		0.53

Values are mean ± SD or n (%).

Included = women still enrolled in the study who had adequate serum in storage to measure thyroid hormone and antibody levels at the 7-year follow-up.

[a]n=254. [b]n=105. [c]n=165. [d]n=139. [e]n=157. [f]n=232. [g]n=164. [h]n=255.

Table 2. Comparison of BPb and thyroid measures by town among study participants.

	BPb (µg/dL)	FT4 (ng/dL)	TSH (µIU/mL)	TPO Ab (IU/mL)
Pristina				
n	147	141	142	147
Mean ± SD	5.57 ± 2.01	1.03 ± 0.16	1.46 ± 0.68	5.12 ± 6.38
Range	1.60-18.60	0.67-1.79	0.20-4.14	1.00-66.33
Mitrovica				
n	144	138	136	144
Mean ± SD	20.00 ± 6.99	0.91 ± 0.17	1.46 ± 0.91	15.45 ± 33.08
Range	5.40-41.30	0.48-1.30	0.20-7.46	0.69-256.65
p-value (ANOVA)	<0.0001	<0.0001	0.99	0.0002

Table 3. Unadjusted and adjusted regression coefficients (for free T4, ln-transformed TSH, and ln-transformed TPO Ab) and odds ratios (for TPO Ab ≥ 10 IU/mL versus < 10 IU/mL) for associations with ln-transformed mid-pregnancy blood lead concentrations, Pristina and Mitrovica combined.

Outcome	Unadjusted R^2 (n)	Unadjusted β or OR (95% CI)	p-value	Adjusted R^2 (n)	Adjusted β or OR (95% CI)[a]	p-value
Free T4 (ng/dL)	0.11 (279)	-0.079 (-0.11, -0.052)	<0.0001	0.25 (277)	-0.074 (-0.10, -0.046)	<0.0001
Ln-TSH (μIU/mL)	0.00027 (278)	-0.012 (-0.098, 0.074)	0.79	0.046 (276)	0.026 (-0.065, 0.12)	0.58
Ln-TPO Ab (IU/mL)	0.075 (291)	0.34 (0.20, 0.48)	<0.0001	0.094 (291)	0.31 (0.17, 0.46)	<0.0001
TPO Ab ≥ vs. < 10 IU/mL	0.062 (291)	2.51 (1.62, 3.89)	<0.0001	0.074 (291)	2.41 (1.53, 3.82)	0.0002

[a]Model covariates: Free T4 = height, ethnicity, BMI, fetal gestational age, maternal education, adults per room; TSH = hemoglobin, ethnicity, BMI, fetal gestational age, maternal age; TPO Ab (continuous and dichotomous) = ethnicity, fetal gestational age, maternal age, adults per room

Figure legends

Figure 1. Recruitment and participation of study subjects.

Figure 2. Scatterplots of measured values for each outcome according to BPb (μg/dL). A) Free T4 adjusted for height, ethnicity, BMI, fetal gestational age, maternal education, adults per room. B) TSH adjusted for hemoglobin, ethnicity, BMI, fetal gestational age, maternal age. C) TPO Ab adjusted for ethnicity, fetal gestational age, maternal age, adults per room. o = Pristina, + = Mitrovica.

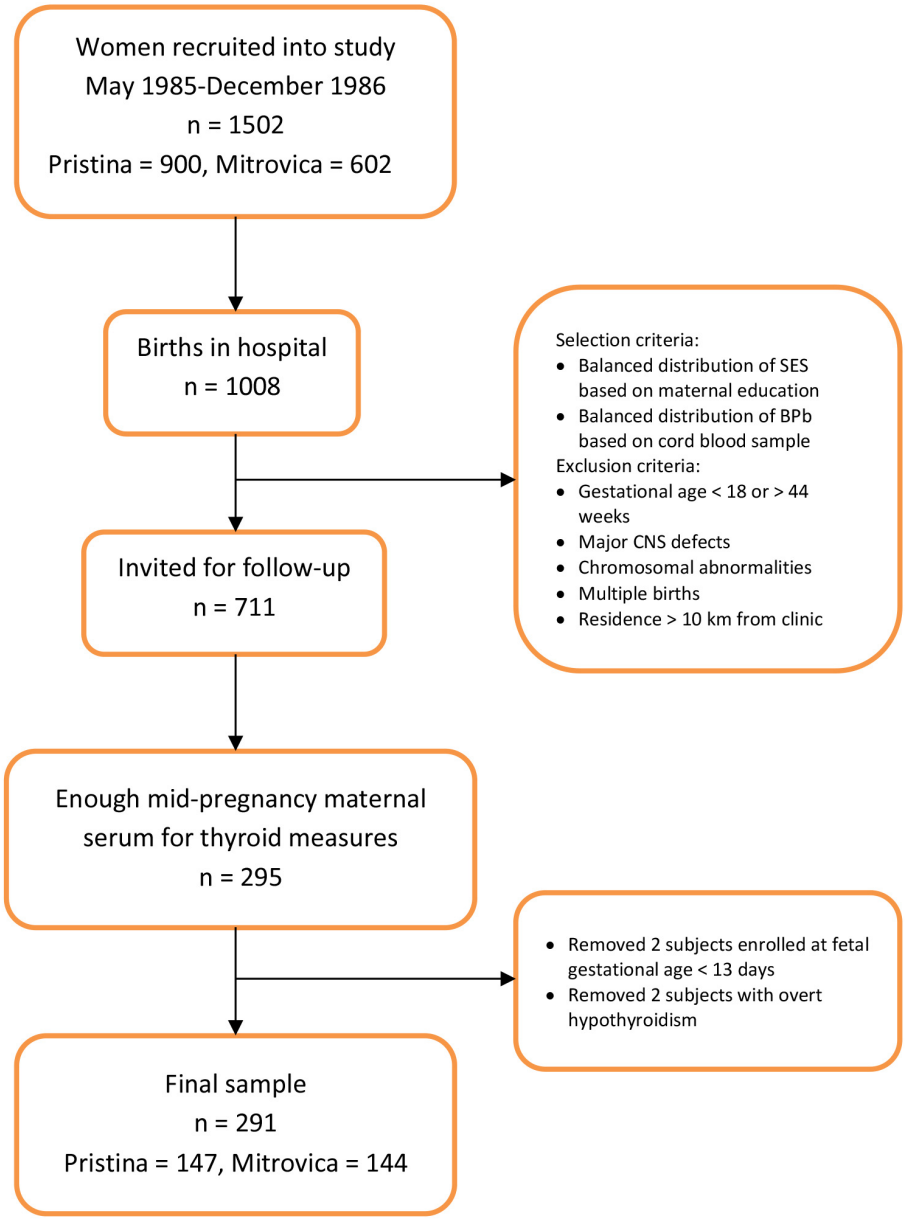
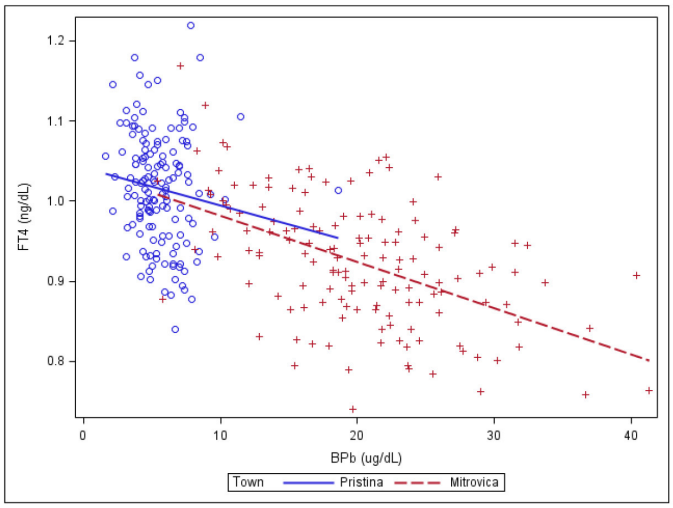
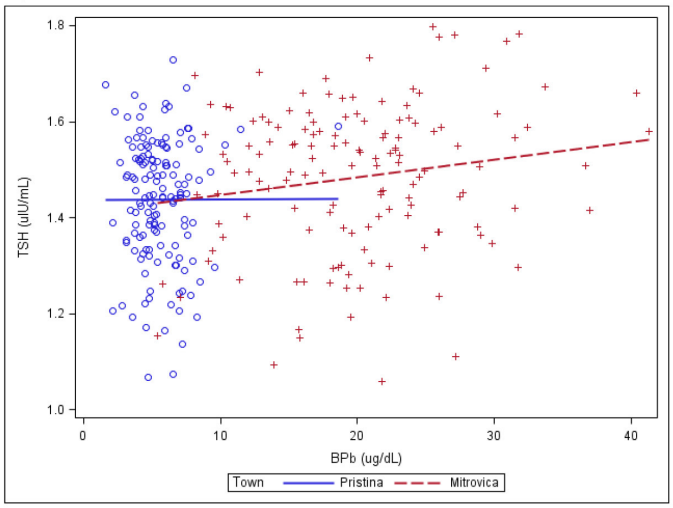

Women recruited into study
May 1985-December 1986
n = 1502
Pristina = 900, Mitrovica = 602
Births in hospital
n = 1008
Selection criteria:
Balanced distribution of SES based on maternal education
Balanced distribution of BPb based on cord blood sample
Exclusion criteria:
Gestational age < 18 or > 44 weeks
Major CNS defects
Chromosomal abnormalities
Multiple births
Residence > 10 km from clinic
Invited for follow-up
n = 711
Enough mid-pregnancy maternal serum for thyroid measures
n = 295
Removed 2 subjects enrolled at fetal gestational age < 13 days
Removed 2 subjects with overt hypothyroidism
Final sample
n = 291
Pristina = 147, Mitrovica = 144

Figure 1

A)


FT4 (ng/dL)
1.2
1.1
1.0
0.9
0.8
0
10
20
30
40
BPb (ug/dL)
Town
Pristina
Mitrovica

B)


TSH (uIU/mL)
1.8
1.6
1.4
1.2
1.0
0
10
20
30
40
BPb (ug/dL)
Town
Pristina
Mitrovica

C)

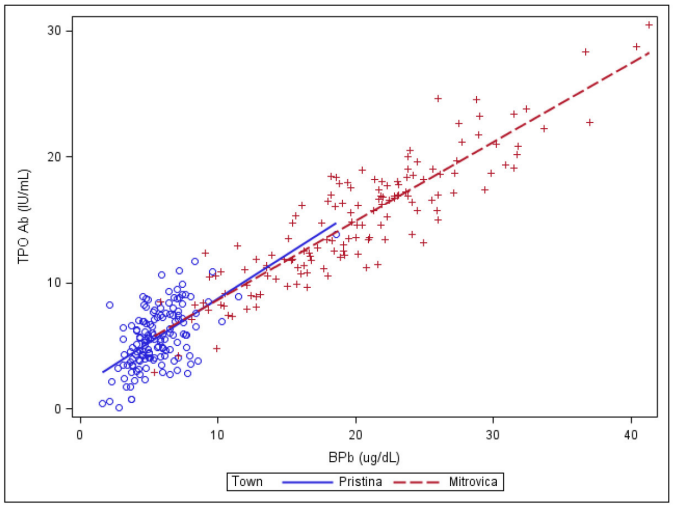

TPO Ab (IU/mL)
30
20
10
0
0
10
20
30
40
BPb (ug/dL)
Town
Pristina
Mitrovica

Figure 2